tion by reducing the surface expression of KDR on HUVECs, or the affinity or total amount of VEGF binding to KDR on HUVECs. Instead, it appears that the durable effect of SU5416 may be due to a residual pool of inhibitor, which is concentrated in cells, that remains associated with cells. The sub-cellular localization and kinetics of elimination of the inhibitor are currently under investigation.

#2843 INHIBITION OF NF-κB BY A NOVEL PROTEASOME INHIBITOR AND ANTI-TUMOR ACTIVITY IN SQUAMOUS CELL CARCINOMA. J B Sunwoo, Z Chen, G Dong, C V Crowl-Bancroft, N Yeh, J Adams, J Mitchell, E Sausville, and C Van Waes, *Leukosite, Inc, Cambridge, MA, and National Inst of Health, Bethesda, MD*

Squamous cell carcinoma (SCC) of the head and neck has an elevated constitutive activation of the NF-κB transcriptional regulator. We have evidence suggesting that this activation is important for cell survival, tumor development, and protection from ionizing radiation. Activation of NF-κB depends on the proteolysis of the inhibitory protein IκB by the 26S proteasome. In this study, a novel proteasome inhibitor, PS-341 (Leukosite, Inc.), was used to inhibit NF-κB, and its anti-tumor effects were examined in a variety of murine and human SCC cell lines. A 50% inhibition of NF-κB was demonstrated by reporter gene and electrophoretic mobility shift assays at 10^{-8} M concentration. This correlated with anti-proliferation assays, demonstrating an IC_{50} of 10^{-8} M. Flow cytometry was used to show that cytotoxicity was preceded by a cell cycle block at the G2/M transition. Anti-tumor activity was also examined *in vivo*, and a significant dose-dependent response was observed. Because exposure to PS-341 induced a cell cycle block at G2/M and was also found to inhibit induction of NF-κB by ionizing radiation, we examined the utility of this compound as a sensitizer to ionizing radiation. We found a 30% increase in radiosensitivity by colonogenic assay after accounting for direct cytotoxic effects of the compound. These results suggest that the use of proteasome inhibitors to target the inhibition of NF-κB may be a useful therapeutic strategy in patients with squamous cell carcinoma of the head and neck.

#2844 RESPONSE OF HUMAN MELANOMAS TO 17-AAG IS ASSOCIATED WITH MODULATION OF THE MOLECULAR CHAPERONE FUNCTION OF HSP90. Angelika Maria Burger, Edward A Sausville, Richard F Camalier, David J Newman, and Heinz H Fiebig, *National Cancer Inst, Bethesda, MD, Tumor Biology Ctr, Freiburg, Germany, and Univ of Freiburg, Freiburg, Germany*

17-allylaminogeldanamycin (17-AAG, NSC 330507) is a new antitumor agent identified by the NCI which has entered phase I clinical trials in the US. Antitumor activity of geldanamycins has been described to result from degradation of signaling proteins and nuclear hormone receptors by binding their molecular chaperone Hsp90. In this study, two human melanoma xenografts, the 17-AAG sensitive MEXF 276 (T/C = 6%), the resistant MEXF 514 (T/C = 60%), and cell lines derived thereof, were chosen to elucidate 17-AAG effects on its potential target Hsp90 and down-stream effector proteins in a time and concentration dependent manner. Tumor tissues were collected after 48h, 72h, and 10d under 17-AAG treatment (at MTD = 80mg/kg/d, for 2x Qdx5). Cell lines were exposed to drug concentrations which cause total growth inhibition (TGI = 375nM in MEXF 276L, 10μM in MEXF 514L cells). By using immunohistochemistry and Western blot analysis we found Hsp90 abundantly expressed in 17-AAG responsive MEXF 276 tumors, but at lower levels in resistant MEXF 514 and in normal tissues. Moreover, whilst 17-AAG treatment did not affect Hsp90 expression in MEXF 514, it caused a rapid decline of Hsp90 in MEXF 276 cells. In latter, this was accompanied by translocation of Hsp90 from cytoplasm and nuclei to cell membranes. In contrast, Hsp72 levels were not changed in either melanoma. As a result of Hsp90 depletion in MEXF 276L cells, down-regulation of Raf-1 and HER-2/neu was observed 8h after drug addition. In MEXF 276 tissues, decrease of Hsp90 was further associated with occurrence of apoptosis. The apoptotic index rose from 9% (48h) over 12% (72h) to 45% (10d) under drug treatment. Our data suggest that the efficacy of 17-AAG is related to its ability to inhibit Hsp90 chaperone function.

#2845 ANTICANCER EFFECTS OF LIPOSOME-ASSOCIATED L AND D STEREOISOMERS OF ET-18-OCH_3. I. Ahmad, G. R Masters, J. Nguyen, J. J Schupsky, A. S Janoff, and E. Mayhew, *The Liposome Company (TLC), Princeton, NJ*

TLC ELL-12 is a liposome based formulation of ET-18-OCH_3 (1-O-octadecyl-2-O-methyl-*sn*-glycero-3-phosphocholine), and is currently in Phase I clinical trials. The L isomer of ET-18-OCH_3 is the active ingredient of ELL-12. We have previously shown the therapeutic efficacy of ELL-12 against several experimental mouse tumors. The aim of the present investigation was to determine any difference in toxicity or therapeutic efficacy of ELL-12 when formulated with L or D stereoisomers of ET-18-OCH_3. The L isomer liposome formulation of ELL-12 significantly reduced toxicity compared to the D isomer liposome formulation when administered once daily, i.v. x 5. L and D isomer formulations of ELL-12 were found to be equally effective in prolonging mean survival time against P388 murine leukemia. However, the L isomer liposome formulation, when administered against established B16/F10 lunge tumors, significantly ($p < 0.05$) reduced the mean number of tumor nodules when compared to control or the D isomer liposome formulation. These studies indicate that ELL-12 formulated with the L isomer of ET-18-OCH_3 is less toxic and more effective against B 16/F10 tumor than the D isomer liposomes.

#2846 THE APOPTOTIC EFFECT OF LONG-CHAIN FATTY AMINES ON HUMAN PANCREATIC CANCER CELLS IS MEDIATED BY SIGNALING PATHWAYS INCLUDING MAPK FAMILY AND CASPASES. Mizukami Yusuke, H. Ura, T. Obara, T. Izawa, N. Yanagawa, S. Tanno, Y. Fujimoto, and Y. Kohgo, *Asahikawa Med Coll, Hokkaido, Japan*

Farnesyl transferase inhibitor (FTI) is usually ineffective n Ki-ras transformed cells. However, we have shown that farnesylamine (FA), one of FTI could induce apoptosis in Ki-ras transformed fibroblasts and human pancreatic cancer cell lines (Mol Carcinogenesis, 1998). Therefore, we speculated that FA may have an another apoptotic mechanism in addition to the inhibition of farnesylation. Considering the chemical formula of FA, the "long-chain fatty amine (LFA)" structure may have a critical role for this mechanism. In this experiment, we used oleylamine (OA) as LFA and examined the signaling pathways to induce apoptosis in Ki-ras transformed fibroblasts and human pancreatic cancer cell lines. In both cells, apoptosis was induced by OA and JNK activity was increased as well as by FA, but not in parent fibroblast (NIH3T3). Although the OA-induced apoptosis was caspase-dependent, caspase inhibitors did not affect JNK activation. The blockage of JNK activity by dominant negative mutant significantly abrogated the cytotoxic effect of OA and DNA laddering. OA did not act as FTI, but decreased the upregulated ERK activity. In contrast to indispensable effect of JNK in OA-induced apoptosis, attenuated ERK activity alone was not sufficient, but might be required, because MEK inhibitor PD98059 alone did not induce apoptosis. The kinase activity of Akt, which transduce p21 ras mediated survival signaling, resulted in no marked change. Multiple signaling pathways including JNK, ERK, and their downstream caspases mediate the apoptosis and might be shared, at least in part, in FA-induced selective cytotoxicity on Ki-ras mutant cells.

#2847 PHARMACOLOGICAL INDUCTION OF PHOSPHATIDYLINOSITOL ACCUMULATION IS ASSOCIATED WITH CYTOLYSIS OF NEOPLASTIC CELLS. Robert E Finney, E Nudelman, S A Shaffer, T White, S Bursten, L L Leer, N Wang, D Waggoner, J W Singer, and R A Lewis, *Cell Therapeutics, Inc, Seattle, WA*

De novo phospholipid biosynthesis is required for growth of tumor cells. Here, we demonstrate that phospholipid biosynthesis through phosphatidic acid (PA) in neoplastic cells can be exploited for development of cytotoxic anti-cancer agents. PA is a key intermediate for biosynthesis of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) through a diacylglycerol (DAG) intermediate and for biosynthesis of the anionic phospholipids, cardiolipin (CL) and phosphatidylinositol (PI), through a cytidinediphosphate-DAG intermediate. In addition to de novo PA production from lysophosphatidic acid (LPA), production of PA by phospholipase D has been cited among the effects of certain oncogenes (e.g. ras, fps, and src) and growth factors (e.g. PDGF, EGF, FGF, Insulin). CT-2584, a cancer chemotherapeutic drug candidate currently in Phase II clinical trials, decreased utilization of PA for PC biosynthesis and increased PA utilization for PI biosynthesis. A two to three-fold increase in PI was observed in tumor cell lines derived from breast, lung and prostate, was associated with cytotoxic concentrations of CT-2584, and occurred well prior to cytolysis of the tumor cell lines. In contrast, cytotoxic concentrations of cisplatin did not induce accumulation of PI, indicating that PI elevation by CT-2584 was not a general consequence of chemotherapy-induced cell death. Consistent with this mechanism of action, propranolol, an inhibitor of phosphatidic acid phosphohydrolase and PC biosynthesis, was also cytotoxic to tumor cell lines, induced PI accumulation, and was synergistic with CT-2584 in cytotoxicity assays. As expected from the biophysical properties of anionic phospholipids on cellular membranes, CT-2584 cytotoxicity was associated with disruption and swelling of endoplasmic reticulum and mitochondria. We conclude that CT-2584 effects a novel mechanism of action involving modulation of phospholipid metabolism in cancer cells.

#2848 THE EFFECTS OF LYSOPHOSPHATIDYLCHOLINE ON TNF-α PRODUCTION INDUCED BY LIPOSOMAL ET-18-OCH3. Marina Y Pushkareva, Andrew S Janoff, and Eric Mayhew, *The Liposome Co , Inc, Princeton, NJ*

The incorporation of 1-*o*-octadecyl-2-*o*-methyl-*sn*-glycero-3-phosphocholine (ET-18-OCH3) into optimized liposomes (ELL-12) overcomes the non-specific hemolytic effects of ET-18-OCH3 while maintaining or enhancing anti-cancer efficacy. ELL-12 is currently in Phase I clinical trial. We showed previously that *in vitro* ELL-12 induced growth inhibition is associated with a time- and dose-dependent production of tumor necrosis alpha (TNF-α). As lysophosphatidylcholine (lysoPC) has been shown to modulate the growth inhibiting effects of ELL-12, it was of interest to determine the effects of lysoPC on ELL-12-induced TNF-α production by U-937 cells. We treated U-937 cells with different concentrations of ELL-12 and lysoPC for various times. Maximum of TNF-α production (0.78±0.17 ng per 10^6 cells) was observed after 48 hours of incubation of U-937 cells with 3-4 μM ELL-12. LysoPC prevented induction of TNF-α production in dose-dependent manner. For example, 20 μM of lysoPC completely prevented TNF-α production at 48 hours, whereas 2 μM lysoPC produced 50 % inhibition. The effects on TNF-α production were not directly coupled to the effects of lysoPC on reduction of ELL-12-induced growth inhibition, since 2 μM lysoPC did not significantly affect ELL-12-induced growth inhibition. ET-18-OCH3 and lysoPC share structural similarity and have common cellular targets including inhibition of *de novo* phosphatidylcholine synthesis. The possible mechanism of inhibition of ELL-12-induced TNF-α production by lysoPC will be discussed.